

## REMARKS

In an Office Action mailed on August 12, 2004 in the above-identified application, currently pending Claims 1-4, 6-13, 15-22, 24 and 25 were rejected. Specifically, the Office Action has substantively rejected Claims 1-4, 6, 13, 15-22, 24 and 25 under 35 U.S.C. §112, first paragraph (enablement); and Claims 1, 6, 7, 9, 10, 15, 16, 18, 19, and 24-25 under 35 U.S.C. §102(b). New Claims 26 to 32 have been added. In light of the amendments above, the enclosed Declaration under 37 CFR §1.132, and the arguments below, applicants respectfully request reconsideration.

### Claim Objections – 35 U.S.C. §112; First Paragraph

Claims 1-4, 6, 13, 15-22, 24 and 25 stand rejected under 35 U.S.C. 112, first paragraph, as failing to comply with the enablement requirement. The Examiner asserts that the terms “ $\alpha$ -proteobacteria” and “plants” in the claims are overly broad in scope. At the outset, applicants respectfully submit that the scope of the term “plant” has already been limited to plant species that are susceptible to crown gall disease. Furthermore, the scope of the term “ $\alpha$ -proteobacteria” has also been limited to bacteria capable of controlling crown gall disease, which have been genetically engineered to express a *trf* operon and produce trifolitoxin for controlling crown gall disease. Therefore applicants do not believe that further narrowing of the scope of these terms is necessary to fulfill the enablement requirement.

However, to expedite prosecution on the merits and to clarify the issue of whether the terms  $\alpha$ -proteobacteria and plants are unduly broad in view of the results presented in the application as filed and to provide evidence that taxonomically divergent plant species are responsive to the biological control of crown gall via various strains of  $\alpha$ -proteobacteria, applicants have submitted herewith the Declaration of Dr. Eric W. Triplett. Dr. Triplett is a highly qualified practicing scientist in this field. (See Exhibit A, Dr. Triplett’s curriculum vitae).

Applicants note that the Declaration, along with the attached Exhibits illustrate recent laboratory experiments conducted by the inventors showing trifolitoxin-producing bacteria was able to prevent crown gall disease on two different cultivars of grapes. The results of these experiments have been summarized in Table 1 of the Declaration. Also, copies of photographic images illustrating control or inhibition of crown gall disease on a variety of grapes have been enclosed with the Declaration as Exhibits B-G.

Table 1 shows that a non-recombinant trifolitoxin-producing strain, *Rhizobium leguminosarum* bv. trifolii T24, can reduce crown gall formation. Furthermore, the data demonstrate that other members of the rhizobiaceae family, such as for example, *R. leguminosarum* CE3 (pT2TFXK) and *Agrobacterium* are also able to successfully control crown gall disease. The results of Table I illustrate that the applicants have been able to show biological control of crown gall with more than one strain of bacteria. Crown gall can be controlled by *R. leguminosarum* CE3 (pT2TFXK), *Rhizobium leguminosarum* bv. trifolii T24, and *Agrobacterium vitis* F2/5 (pT2TFXK); which demonstrate the efficacy of the methods of the invention beyond simply the *Nicotiana glauca* plant using the *Agrobacterium* strain. Applicants have shown that the inventive method is applicable to all plants, particularly because crown gall disease occurs when the soil bacterium, such as an  $\alpha$ -proteobacteria enters the stem of the plant through a wound site. Therefore, applicants should not be limited to using the methods of the invention for biologically controlling crown gall on only grape plants, fruit trees, and rose plants; but instead on all crown gall susceptible plants, as described in Dr. Triplett's Declaration.

To further facilitate prosecution on the merits, applicants respectfully submit that independent Claims 1, 10 and 19 have been amended for clarification purposes. In Claim 1 the  $\alpha$ -proteobacteria has been limited to a strain of Rhizobiaceae bacteria which is capable of producing trifolitoxin. Furthermore, Claim 10 has been amended to include two specific types of  $\alpha$ -proteobacteria, *Rhizobium* and *Agrobacterium* bacteria, both of which can produce trifolitoxin. Claim 19 has also been amended to include a strain of *Agrobacterium*. Support for these amendments can be found for example at paragraphs: [0054-0059]; and [0024], [0034]; and Table 1, [0044] of the specification. Accordingly, applicants have canceled dependent Claims 11, 14, 20 and 23. No new matter has been added with these claim amendments. Therefore, applicants respectfully request that in view of these claim amendments and the additional results presented in the Declaration, the rejection should be reconsidered and withdrawn.

#### Claim Rejections – 35 U.S.C. §102

Claims 1, 7, 9, 10, 16, 18, 19, and 24-25 stand rejected under 35 U.S.C. 102(b) as being anticipated by Robleto, et al. Environmental Microbiology, (1998), Vol. 64, No. 7, page 2630-2633. The Examiner states that the prior art teaches a genetically engineered  $\alpha$ -proteobacteria comprising a pT2TFXK plasmid, and so anticipates product Claims 19 and 25

since the identical strain would have the inherent property to act as a biocontrol agent. Additionally, the Examiner states that the prior art teaches all of the method steps and starting materials of the process Claims 1, 7, 9, 10, 16 and 18 wherein the desired result of crown gall control would be inherent following trifolitoxin production.

In response, applicants respectfully submit that Robleto et al., discloses the DNA sequence analysis, subcloning, and insertional mutation analysis of the region required for TFX production and resistance. However, this disclosure does not teach or suggest a method of controlling crown gall disease by introducing onto a plant a strain of *Rhizobiaceae* engineered to express a TFX operon and produce trifolitoxin. This novel idea was only discovered through the conception and reduction to practice by the inventors, evidenced by the results described in the application and in Dr. Triplett's Declaration.

Applicants submit that there are several key differences between the disclosure of Robleto et al., (i.e., nodulation) and the present invention (i.e., crown gall), such as for example, the different site of infection, different mechanism of infection, etc. As Dr. Triplett stated, with respect to the different site of infection, it is well known in the art that rhizobia enter through root hairs in a mechanism that involves highly controlled and coordinated signaling by both the plant and bacteria. The antibiotic has the opportunity to disrupt this signaling at any time. It is also well known in the art that antibiotics that are effective in soil or *in vitro* may not be effective or involved in disease control. (See, for example, Burr et al., "Biological control of grape crown gall with non-tumorigenic *Agrobacterium vitis* strain F2/5," Am. J. Enol. Vitic (1993) 45:213-219; which describes an antibiotic produced by *Agrobacterium vitis* F2/5, which does NOT produce any disease control phenotype.)

Furthermore, applicants submit that their initial attempts to control crown gall disease using bacteriolytic peptides expressed by transgenic plants failed. This may have been due to a turnover of the peptides (i.e., at a rate that does not allow for effective concentrations to be produced). It is believed that this information is directly relevant to TFX production, because it may have been subject to the same rapid peptide turnover in plants. Accordingly, applicants strongly submit that the disclosure of Robleto et al., does not anticipate or render obvious the method claims of the present invention.

Furthermore, with respect to the Examiner's inherency rejection, asserting that the desired result of crown gall control would be inherent following trifolitoxin production, applicants respectfully traverse this rejection. It is well known that:

“To establish inherency, the extrinsic evidence "must make clear that the missing descriptive matter is necessarily present in the thing described in the reference, and that it would be so recognized by persons of ordinary skill."  
Continental Can Co. v. Monsanto Co., 948 F.2d 1264, 1268, 20 U.S.P.Q.2d 1746, 1749 (Fed. Cir. 1991).”

Applicants submit that clearly at the time the application was filed the biological control of crown gall disease via a TFX-producing strain was still unknown and therefore, would not have been so recognized by persons of ordinary skill. Furthermore, applicants submit that the above-noted inherency rejection seems to rely on the assumption that what applies to nodulation improvement would equally work for crown gall control. There is not a 1:1 correlation between biocontrol and *in vitro* antibiotic production. As Dr. Triplett has noted in the attached Declaration, nodulation and galling are very different processes and more importantly occur in very different environments. For example, *Rhizobia* infect root hairs; in contrast *Agrobacteria* infect wounded or de-differentiated (e.g., callus culture) plant tissue.

Dr. Triplett has also recited a number of publications that demonstrate *in vitro* antibiotic activity is neither necessary nor sufficient for biocontrol in plants. (See, for example: Burr, T.J., et al., “Biological control of grape crown gall by strain F2/5 is not associated with agrocin production or competition for attachment sites on grape cells” *Phytopathology* 1997; 87(7): 705-711; Samac, D.A., et al., “Effects of antibiotic-producing *Streptomyces* on nodulation and leaf spot in alfalfa” *Applied-Soil-Ecology*. 2003; 22(1): 55-66; and Schmiedeknecht, G., et al., “Use of *Bacillus subtilis* as biocontrol agent. V. Biological control of diseases on maize and sunflowers” *Zeitschrift-fuer-Pflanzenkrankheiten-und-Pflanzenschutz*. 2001; 108(5): 500-512.) Applicants note that copies of these publications are provided herewith.

Nevertheless, in the hopes of expediting prosecution on the merits and to obviate any need for an Appeal, applicants have amended Claims 1, 10 and 19, as identified herein above for clarification purposes. Accordingly, applicants submit that in view of the above amendments, the inherency rejection of Claim 19 and 25 will be rendered moot.

Also, claims 1, 6, 7, 9, 10, 15, 16, 18, 19, 23, and 25 stand rejected under 35 U.S.C. 102(b) as being anticipated by Robleto, as applied to Claim 1, 7, 9, 10, 16, 18, 19 and 25 in light of Breil, et al., J. Bacteriol. (1993), vol. 175, pages 3696-3702 and Breil et al., NCBI Accession No. L06719, locus RHMTFXA2G, August 4, 1993.

In response, applicants respectfully submit that in view of the claim amendments made herein to independent Claims 1, 10 and 19, the invention is not anticipated by Robleto et al., and/or Breil et al. As recited earlier herein, neither of these two disclosures alone or combined make a connection between TFX- producing bacteria and the biological control of crown gall disease.

Also, the Examiner states that the TFX operon disclosed in Breil et al., is both necessary and sufficient to code for this peptide toxin. Applicants respectfully submit that Breil et al., does not teach the pT2TFXK plasmid, nor does it make the connection between the production of the trifolitoxin and the ability to biologically control crown gall disease. Furthermore, given the disclosure of Burr et al., referred to earlier herein above, which teaches against gall control with an *in vivo* active antibiotic and shows that there is not a direct correlation between antibiotic production and biological control of crown gall disease; applicants believe that Breil et. al., is not sufficient to anticipate or render obvious the invention. Therefore, applicants submit that the disclosures of Robleto et al., and Breil et al., either alone or in combination would not provide any motivation to one of ordinary skill in the art to use TFX- producing bacterial strains to biologically control crown gall disease.

#### New Claims

Applicants have respectfully added new Claims 26-32. New Claims 26 and 27 which depend from Claims 1 and 10, respectively, now recite a species of *Rhizobiaceae*, support for which can be found in the Examples at paragraphs [0021, 0024, 0034, 0050, etc.] of the specification. Claim 28 and dependent Claims 29-32 recite a method for controlling crown gall disease wherein a crown gall susceptible plant and an effective amount of trifolitoxin producing bacterium are provided. The trifolitoxin producing bacterium is then applied onto the plant in a bacterial suspension which is sprayed onto the crown of the susceptible plant. Support for these new claims can be found for example in the examples at paragraphs [0044, 0045, and 0049] of the specification.

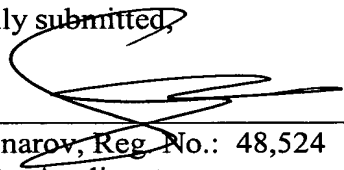
Finally, with respect to the Examiner's remarks about Claims 2-4, 8, 11-13, 17 and 20-22 being deemed free of the prior art, applicants respectfully re-submit that the reason for such a determination should not be because the prior art does not teach *Agrobacterium* or *Agrobacterium vitis* F2/5 as bacterial host for trifolitoxin production, but rather it is more fair to say that the prior art does not teach or even suggest the biological control of crown gall disease with a TFX-producing strain. Applicants respectfully submit that production of

antibiotic does not equal gall control. (See Burr et al., recited hereinabove). This reference shows that *A. vitis* F2/5 antibiotic is effective against *A. vitis* strains *in vitro*. However, this antibiotic does not provide gall control in a susceptible (e.g., wounded) plant.

Accordingly, in view of the above claim amendments and remarks, the application is now believed to be in condition for allowance. Applicants respectfully request that a timely Notice of Allowance be issued in this case.

Applicants have also included a fee for the addition of new Claims 26-35. No other fee is believed to be due in connection with this response. However, if any fee is due in this or any subsequent response, please charge the fee to the same Deposit Account No. 17-0055.

Respectfully submitted,



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